The $\alpha\beta$ -Elimination of L-Serylglycine O-Sulphate and Related Dipeptides

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The non-enzymic degradation of L-serine O-sulphate to pyruvate, ammonia and SO_4^{2-} may be conveniently catalysed by pyridoxal or pyridoxal phosphate and metal ions (Thomas, Dodgson & Tudball, 1968). This reaction is typical of a number of similar $\alpha\beta$ -elimination reactions that occur with appropriate β -substituted amino acids (see Longenecker & Snell, 1957). However, no reports have been made on the application of this reaction to peptide-bound forms of such amino acids. Thomas *et al.* (1968) reported briefly on the ability of pyridoxal and Cu^{2+} ions to catalyse the non-enzymic desulphation of L-serylglycine O-sulphate and studies on this reaction have now been extended.

On incubation of L-serylglycine O-sulphate at 50°C with pyridoxal and cupric chloride, the products obtained were SO_4^{2-} , ammonia and an oxo acid. The oxo acid was isolated by chromatography on a strongly basic resin (De-Acidite FF-IP) by using the conditions described by Dixon (1964) and was characterized as N-pyruvoylglycine by comparison with an authentic synthetic sample. The comparison involved mixed-melting-point determination and electrophoretic and spectral examination (Errera & Greenstein, 1947). N-Pyruvoylglycine was also detected as a product of the breakdown of L-serylglycine in this system, but the presence of the electronegative sulphate group increased the rate of degradation eightfold.

Spectral changes observed during the pyridoxal-catalysed degradation of L-serylglycine or its sulphate ester were consistent with the formation and subsequent breakdown of a cupric chelate of a Schiff-base complex with an absorption maximum at 384 nm. Evidence was obtained for the formation of a Schiff-base complex between pyridoxal and glycyl-L-serine O-sulphate, but no degradation products of this dipeptide could be detected, indicating the necessity of a free amino group on the serine residue for breakdown of the complex to occur.

Tudball, Thomas & Fowler (1969) have described a rat liver enzyme that catalyses a pyridoxal phosphate-independent $\alpha\beta$ -elimination of L-serine O-sulphate. By using a highly purified preparation of this enzyme, the degradation of a number of dipeptides containing L-serine O-sulphate as the N-terminal residue has been demonstrated. As in the non-enzymic system, N-pyruvoylglycine was a

product of the breakdown of L-serylglycine O-sulphate. Similar peptides of pyruvate and the corresponding α -amino acid were obtained from the other dipeptides investigated. The rate of degradation of these dipeptides decreased with the increasing molecular size of the C-terminal amino acid, presumably reflecting steric hindrance. The enzymic mechanisms responsible for these pyridoxal phosphate-independent $\alpha\beta$ -eliminations are currently under investigation.

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The State of Pigment-Epithelium Lysosomes in the Retina of Normal Rats and those with Retinitis Pigmentosa

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Campbell albino rats, which are homozygous for the retinitis pigmentosa allele (retinal dystrophy), become blind at 3–4 weeks (Dowling & Sidman, 1962); the histology of the retina becomes abnormal at about 14 days, with gross disorganization of the rods developing at 18 days (Lucas, Attfield & Davey, 1955). The object of the present study was to determine whether this degeneration was related to changes in the lysosomes of the pigment epithelium, which is in close apposition to the rods. Albino Wistar rats, which showed no spontaneous degenerative changes in any of the eye tissues, were used as controls. Four normal and four 'dystrophic' animals were killed at an age of 1 week; a similar number at 2 weeks; and two normal and two 'dystrophic' animals were taken at 4, 6 and 9 weeks of age. The eyes were removed, chilled at -65°C and sectioned at $10\mu\text{m}$ at -25°C . The sections were incubated for naphthylamidase and for acid phosphatase activity (Chayen, Bitensky, Butcher & Poulter, 1969).

The latter was used to indicate the fragility of the lysosomal membrane by determining the incubation time needed to produce an appreciable reaction with β -glycerophosphate in the pigment-epithelium

cells (Bitensky, 1963). 'Freely available' naphthylamidase activity was measured by microdensitometry of the coloured end product (Chayen, Bitensky & Poulter, 1970) in this region of the retina. Total naphthylamidase activity was taken as the maximum activity in unit time with or without various periods of pretreatment in 0.1M-acetate buffer, pH 5.0, at 37°C. The 'freely available to total' ratio was taken as an indication of lysosomal membrane stability.

At 1 week there was little difference in the acid phosphatase reaction. At this age the naphthylamidase reaction showed the lysosomes to be fully permeable in 'dystrophic' eyes but normal retinas (three out of four) required acid pretreatment to disclose full activity. In 2 week-old animals this difference was more marked and was confirmed by the results with acid phosphatase. This difference in stability of the lysosomal membrane in the pigment epithelium was found consistently, by both tests, in 6- and 9-week-old animals.

It seems probable therefore that a defect of lysosomal membranes precedes histological degeneration in this condition. This would accord with the previous observation (Reading, 1966) that an abnormal local concentration of retinol occurs in the pigment epithelium at 12 days of age, as a result of an anomalous visual-cycle mechanism.

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Some Studies on the Biosynthesis of Tocochromanols

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Rubber latex contains 8-methyl-, 7,8-dimethyl- and 5,7,8-trimethyl-tocotrienol (δT_3 , γT_3 and αT_3 respectively) together with α -tocopherol (αT), and the biosynthetic pathway $\delta T_3 \rightarrow \gamma T_3 \rightarrow \alpha T_3 \rightarrow \alpha T$ is indicated (Dunphy, Whittle, Pennock & Morton, 1965). Plant leaves on the other hand have predominantly αT , with smaller amounts of γT and

occasionally δT but no tocotrienols. Since methionine is incorporated into α - and γ - but not δ -tocochromanol (Whittle, Audley & Pennock, 1967; Threlfall, Whistance & Goodwin, 1968) and [β - ^{14}C]tyrosine is incorporated into all tocochromanols it has been proposed that homogentisate supplies the aromatic ring and 8-methyl group of tocochromanols (Whistance & Threlfall, 1967). *p*-Hydroxyphenylpyruvate has been shown to be a precursor of tocochromanols in latex and leaves of *Hevea brasiliensis*, as is homogentisate also.

Hevea leaves were incubated with [U - ^{14}C]homogentisate (1 μ Ci) and radioactivity was detected in αT (1680 c.p.m.), γT (13160 c.p.m.) and δT (440 c.p.m.), and in a similar experiment with *p*-hydroxy[U - ^{14}C]phenylpyruvate (3.9 μ Ci) the radioactivities were αT (4900 c.p.m.), γT (19800 c.p.m.) and δT (800 c.p.m.). The amounts of tocopherols present in the leaves were in the ratio αT : γT : δT , 4:2:1 and it is apparent that γT could be a precursor of αT but δT is not a precursor of γT , which agrees with the data of Threlfall & Whistance (1968).

With a cell-free system such as latex the incorporations were much lower but from homogentisate (1.1 μ Ci) radioactivity was found in αT_3 (160 c.p.m.), γT_3 (630 c.p.m.) and δT_3 (450 c.p.m.) with a small amount in αT , and from *p*-hydroxyphenylpyruvate αT_3 (45 c.p.m.), γT_3 (1020 c.p.m.) and δT_3 (400 c.p.m.), again with a trace in αT . Latex contains relatively huge amounts of γT_3 and thus specific radioactivities mean very little, but clearly the results may be consistent with the pathway $\delta T_3 \rightarrow \gamma T_3 \rightarrow \alpha T_3 \rightarrow \alpha T$.

Thus although sequential methylation of tocotrienols is likely in latex a similar tocopherol sequence is unlikely in leaves.

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